Docket No.: 09600-00031-US
(PATENT)

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Patent Application of:
Heinz Schneider

Application No.: 10/538,223 | Confirmation No.: 9409

Filed: June 29, 2005 | Art Unit: 1655

For: FORMULATION WHICH CAN BE ADMINISTERED GASTROINTESTINALLY, AND THE USE THEREOF | Examiner: M. L. McCormick

Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Dear Sir:

DECLARATION OF DR. HEINZ SCHNEIDER

I, Heinz Schneider, hereby declare:

1. I am the inventor of the instant application Serial No. 10/538,223.

2. I am currently employed by HealthEcon AG, Basel, Switzerland as Managing Director

3. I reside at Bulliard 50, CH-1792 Cordast, Switzerland; I am a citizen of Switzerland.

4. I am familiar with subject matter and the prosecution of application Serial No. 10/538,223. The claims of application Serial No. 10/538,223, as presently presented, are directed to methods for averting or reducing the risk of postoperative complications by gastrointestinally administering to a surgical patient, less than twenty-four hours before a surgical procedure, a composition comprising a) green tea extract and b) at least one NO donor which is a substrate of NO synthetase, and/or one precursor of this NO donor, wherein the NO donor and precursor are selected from the group consisting of glutamine, precursors of glutamine, trinitroglycerin, isosorbite dinitrate, nitroprussite, aminoguanidine, spermine-NO,

spermidine-NO and SIN 1 (3-morpholinosydnone imines), or the physiologically tolerated salts or combinations thereof. The foregoing compositions are also claimed in the application.

5. I am familiar with the Office action from the United States Patent and Trademark Office dated April 19, 2007. It is my understanding that the Examiner rejected the claims as obvious because, in the Examiner's view, it was obvious to combine green tea extract and glutamine in a composition for averting or reducing the risk of postoperative complications, since each of the components was known for use in protecting against ischemia/reperfusion injury. Additionally, the Examiner asserted that administering such a composition less than twenty-four hours prior to surgery is routine optimization of the method because it was known to administer the components of the composition before surgery.

6. This declaration is submitted to present data showing a) the usefulness of the claimed compositions and methods for averting or reducing the risk of postoperative complications and b) that the skilled artisan would not had have a reasonable expectation of success in combining green tea extract with glutamine, as combination of other antioxiants with glutamine do not provide a protection against postoperative complications.

7. In a first series I performed experiments on the effects of "PreOP" booster on pigs after warm ischemia to the liver in collaboration with Arash Nickkholgh, Rui Liang, Xiaohai Guan, Zhanqing Li, Markus Zorn, Martha-Maria Gebhard, Steffen Benzing, Markus W. Büchler and Peter Schemmer.

Green tea (Camellia sinenesis) and its polyphenolic constituents have been shown to be potent scavengers of reactive oxygen species. Thus, this study was designed to assess its effects on serum transaminases after warm liver ischemia-reperfusion in pigs.

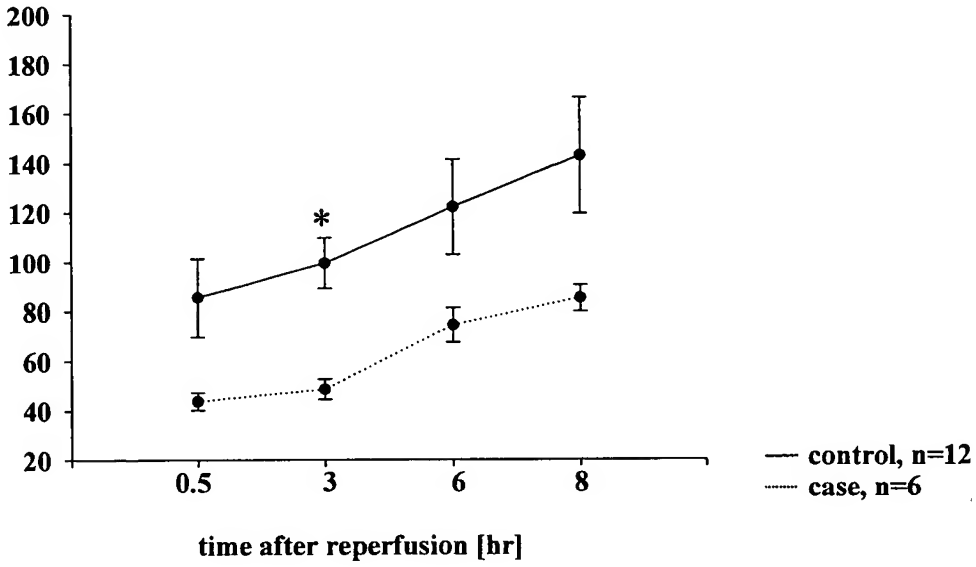
The experiments were performed as follows:

Pigs (German Landrace pigs, 30 kgBW) were treated with PreOP Booster containing green tea extract (GTE; 1000 mg/dose) and glutamine, 3 times (24, 12 and 2 hours) before warm ischemia to the liver. Controls were given isocaloric maltose dextrin with the same flavor. Warm ischemia was induced for 40 minutes to the whole liver. After reperfusion the serum

enzymes AST, a marker enzyme for ischemic liver injury, was measured 30 minutes, 3, 6, and 8 hours after reperfusion to index liver injury.

In both groups, AST increased postoperatively over time following warm ischemia. Oral application of PreOP Booster containing green tea extract (GTE; 1000 mg/dose) and glutamine given 3 times before warm ischemia to the pigs significantly diminished the increase of AST (see Figure below).

AST



values are Mean ± SEM; * : p< 0.05

These data demonstrate that GTE together with glutamine ameliorates ischemia-reperfusion injury in pig livers after warm ischemia. Gastroenterologically the pig is the animal model closest to humans. Thus, the significant effect of PreOP Booster in the pig model is expected to be observed in humans as well and to a similar extent (dose dependent).

Composition of "PreOP Booster":

		Preop booster	
		Dosage per day	
		2 sachets of powder To be dissolved in 500 ml	1 sachet (-70g) To be dissolved in 250 mL
Energy	Kcal	~520	~260
Glutamine	g	30	15
Fat	g	---	---
CHO	g	100	50
Vitamin C	mg	1500	750
Vitamin E	mg	500	260
B-carotene	mg	10	5
Selenium	µg	300	150
Zinc	mg	20	10
Green Tea Extract	g	2	1

8. In a second series I performed experiments on the evaluation of an enteral feeding solution on hepatic injury after hemorrhage / resuscitation to rats in collaboration with Mark Lehnert, Stephen E. McKim, Zihi Zhong, Blair U. Bradford, Gavin E. Arteel and John J. Lemasters.

Gut injury during hemorrhagic shock and resuscitation may be an important contributor to the systemic inflammatory response syndrome and multiple organ dysfunction that often follows hemorrhage and resuscitation. Protection of the gut against hemorrhage/resuscitation-induced damage might therefore lead to a better outcome after hemorrhagic shock. With these experiments the hypothesis was tested that enteral administration of a gut protective S1 solution (as hereinafter defined) can decrease systemic organ damage after hemorrhage/resuscitation.

Methods and composition of S1 solution

Insertion of Gastritic Cannulas

Female Sprague Dawley rats (240-300 g) were anesthetizes with sodium petobarbital (50 mg/Kg i.p.). A small incision was made in the dorsal cervix followed by a vertical midline incision in the sik inof the abdomen from the syphoid cartilage extending to the mid-abdomen. After

laparotomy was performed in the linea alba, the stomach was exposed, and a modified purse-string suture was created through the serosa and musculature of the fore stomach. After making a small opening in the fore stomach at the center of the purse-string, the tip of the gastric cannula was inserted into the fore stomach to the level of the Dacron disk and the purse-string was tied. Four additional sutures secured the disk to the stomach wall. A subcutaneous tunnel was made from the dorsal cervical incision, and the proximal end of the gastric cannula was passed through the tunnel and extended to the dorsal incision. The stomach was replaced into the abdominal cavity. A suture was then passed through the abdominal wall and into the Dacron disk, resulting in a tight fixation of the cannula to the abdominal wall. Both the peritoneal cavity and skin layer were closed wich 3-0 silk sutures. The rat was then placed in a prone position, and the proximal end of the cannula was passed through the musculature of the dorsal cervix. An anchoring button covered wich Dacron felt was sutured to the muscles using 4-0 Prolene. The skin was closed around the button stem with 4-0 Prolene. The total surgical procedure took 30-40 minutes.

Hemorrhagic Shock Model: Seven to 10 days alter the placement of the enteral tube, rats were randomly assigned to experimental groups. In the "Prior to Procedure" group, rats received an enteral bolus (2 ml) of either S1 solution or distilled water. After 2 h, the rats were anesthetized with pentobarbital sodium (35 mg/g body weight, i.p.). Body temperature was monitored using a tele-thermometer (YSI 423s) placed in the colon and maintained at 37°C with warming lamps. Blood pressure was monitored via polyethylene tubing (PE-50) inserted into the right femoral artery using a low pressure analyzer (LPA-200, Digi-Med, Louisville, KY). The left jugular vein was cannulated (PE-50) to administer lactated Ringer's solution and to maintain anesthesia with pentobarbital whenever the comeal reflex reappeared. The right carotid artery was cannulated with polyethylene tubing (PE 50), and shock was induced over 5 min by withdrawing blood into a heparinized syringe until mean arterial pressure decreased to 30-35 mm Hg. Constant pressure was maintained by further withdrawal of small amounts of blond as necessary for 60 minutes. After 60 minutes of hypotension, rats were resuscitated by transfusion of 60% shed blood over about 5 min. Simultaneously, lactated Ringer's solution (twice the shed blond volume) was infused over approximately 1 b. Rats trat were assigned to the "at reperfusion" group received an

enteral bolus (2 ml) of either S1 solution or distilled water alter resuscitation rather than before hemorrhage. In all rats, continuous infusion of either S l solution or water at a rate of 20 ml over 24 hrs was then initiated via a syringe pump. Immediately alter resuscitation, catheters were removed, the vessels were occluded, and wounds were closed. All experiments were performed in adherence to National Institutes of Health guidelines and the use of experimental animals. Protocols were approved by the Animal Use Committee of the University of North Carolina at Chapel Hill.

Blood sampies were collected in some animals before shock immediately alter insertion of catheters to obtain "sham" values. Two hours alter the end of reperfusion, blond was collected for CK and AST analysis. At 18 h alter the end of reperfusion, blood was collected from the portal vein for endotoxin measurement and from the vena cava for enzyme measurements. Carcasses were infused wich normal saline followed by 4% formalin via the portal vein and parts of the distal ileum, liver, lang and kidney were stored in 10% buffered formalin, embedded in paraffin, sectioned and stained wich hematoxylin and eosin.

The experimental design is summarized as follows.

Prior to procedure group: Overnight fast — bolus (water of S1 solution) – shock – resuscitation and start of continuous enteral infusion (water or S1 solution)

At reperfusion procedure group: Overnight fast — shock – resuscitation and bolus (water or S1 solution) followed by start of continuous enteral infusion (water or S1 solution)

Statistics: A t-test or ANOVA was used for statistical comparisons unless otherwise stated.

$P < 0.05$ was considered significant.

The S1 solution used in these experiments had the following composition

			S1
			500 ml
Energy		Kcal	250
		KJ	1050
Caloric density		Kcal/ml	0,5
Caloric distribution			
Protein			72%
CHO			26%
Fat			2%
Osmolarity		mosmol/l	525
Protein,			45
thereof		glutamine	30
		arginine	-
		glycine	10
Total nitrogen		g	9
Fat			
thereof	- SFA *	g	
	> LCT *	g	-
	> MCT *	g	-
	- MUFA *	g	-
	- PUFA *	g	-
	> Linoleic acid	g	-
	> α- Linolenic acid	g	-
	> EPA + DHA	g	-
	- n6/n3 Fatty Acids		-
	- Cholesterol		-
Tributyin		g	1
CHO			16
thereof	- sugars	g	-
	- Lactose	g	-
	- Maltodextrin	g	16
	- Glucose	g	-
Vit. A		mg RE	-
β- Carotene		mg	10
Vit. D3		µg	-
Vit. E		mg TE	500
Vit. K1		µg	-
Vit. B1		mg	-
Vit. B2		mg	-
Niacin		mg	-
Vit. B6		mg	-
Vit. B12		µg	-
Pantothenic acid		mg	-
Biotin		µg	-
Folic acid		µg	-
Vit. C		mg	1500
Choline		mg	-

Sodium	(Na) mg	460
Potassium	(K) mg	-
Chloride	(Cl) mg	-
Calcium	(Ca) mg	-
Phosphorus	(P) mg	-
Magnesium	(Mg) mg	-
Iron	(Fe) mg	-
Zinc	(Zn) mg	20
Copper	(Cu) mg	-
Manganese	(Mn) mg	-
Iodide	(I) µg	-
Fluoride	(F) mg	-
Chromium	(Cr) µg	-
Molybdenum	(Mb) µg	-
Selenium	(Se) µg	300

Results

A. Model evaluation and comparability between groups.

To show the comparability between experimental groups for each group the following was determined:

1. The time interval between enteral tube placement and shock
2. The body weight change between enteral tube placement and shock
3. The body weight at time of shock
4. The mean arterial pressure during shock
5. The operating time for catheter placement, shock, resuscitation, catheter removal (total procedure time)

In these experiments it has been shown that the groups were not statistically different in any of the features 1-5 above.

Outcome variables für the study were:

1. Survival time
2. Creatine kinase (CK) before (sham) and at 2 and 18 h after the end of reperfusion
3. Aspartate aminotransferase (AST) before (sham) and at 2 and 18 h alter the end of reperfusion
4. Alianine aminotransferase (ALT) 18 hrs alter the end of reperfusion
5. Portal vein endotoxin 18 h after the end of reperfusion
6. Histologic evaluation of the gut, liver, lung and kidney 18 h afer the end of reperfusion

The following results were obtained regardings changes in these outcome variables after hemorrhagel/resuscitation to the various treatment groups.

Average time of survival time in treatement groups receiving water or S1 solution prior to hemorrhage/resuscitateion: Time was measured beginning at the initial bolus application of water or S1 and ending at the time of death or sacrifice at 18 hrs after end of the reperfusion. Average survival time was close to 15 h for both water- and S1-treated groups.

Creatine kinase after insertion of catheters (Sham) and 2 h after the end of reperfusion: Differences between the treatment groups were not statistically significant.

Creatine kinase after insertion of catheters (Sham) and 18 h after the end of reperfusion: Differences between the treatment groups were not statistically significant.

AST after insertion of catheters (Sham) and 2 h after the end of reperfusion: Differences between the treatment groups were not statistically significant.

ATS after insertion of catheters (Sham) and 18 h after the end of reperfusion: Differences between the treatment groups were not statistically significant.

ALT at 18 h after the end of reperfusion: Differences between the treatment groups were not statistically significant.

Endotoxin measurement Portal blood was collected 18 hours after the end of reperfusion, and endotoxin was measured by the Limulus assay. Most samples clotted. Of the unclotted samples, one sample in the "water prior to procedure" and two samples in the "solution prior to procedure" group were negative for endotoxin. Three samples in the "solution at reperfusion" group were also negative. Of the remaining clotted samples, one sample from the "water at reperfusion" group had an endotoxin level of 105 pg/ml. The other clotted samples were negative for endotoxin.

Histology. Histological analysis of tissue harvested at 18 h alter the end of reperfusion also failed to reveal differences between groups. In all groups, gut architecture was normal, and inflammatory changes were not observed. TUNEL staining of nuclei was relatively rare and was similar in all four groups. In the liver, coagulative necrosis was prominent with increased cellularity and vacuolization of hepatocytes as other indices of acute damage, but no group differences could be discerned. In the lung, thickening and increased cellularithy of the alveolar wall was the prominent finding, but again no differences between groups were observed. In the kidney, histopathological changes were mild to moderate with some proximal tubular necrosis and formation of eosinophilc casts. No group differences were observed.

Summary for experiments of second series

1. All 4 groups were comparable wich respect to the interval between enteral tube placement and shock, the body weight change between enteral tube placement and shock, the body weight at time of shock, the mean arterial pressure during shock, and operating time for catheter placement, shock, resuscitation, catheter removal (total procedure time).

2. The absence of clinical signs of infection and normal enzyme values for AST and CK in the "sham" rats show that the operation for gastric cannula placement was well tolerated without significant injury.

3. Application of either S 1 solution or water 2 h "prior to procedure" showed a weak but non-significant tendency towards an elevated value of the creatine kinase at 2 h after the end of reperfusion compared to the administration ofwater or SI solution "at reperfusionr".

4. Rats treated with water or S1 solution either "prior to procedure" or "at reperfusion" did not show any statistical significant differentes in enzyme measurements.

5. Histologic evaluation of organ damage at 18 h after the end of reperfusion showed comparable changes in all groups. In the liver, typical midzonal and periportal damage (coagulative necrosis, vacuolization) was observed. The lungs showed thickening of the alveolar wall with increased cellularity. Pathological changes in the kidney were mild and manifested by small areas of necrosis of proximal tubules, some cast formation and mild parenchymal bleeding. The gut did not show any pathological changes at 18 h alter the end of reperfusion. The lack of changes in the gut may reflect an effective regenerative response to the injury or the absence of gut injury alter the shock procedure.

Conclusion

Enteral application of S1 solution did not elicit a beneficial effect in rats subjected to hemorrhagic shock and resuscitation in comparison to water.

9. Based on the above described experiments with administration of a solution containing large amounts of antioxidative vitamins and glutamine and with administration of a solution of GTE, as antioxidative compound, and glutamine is not expected to demonstrate a liver protective effect in a situation involving ischemia-reperfusion injury such as hemorrhagic shock or warm ischemia. Therefore the observed beneficial effect of a combination of GTE and glutamine was an unexpected, i.e. surprising finding. Likewise, the notion that the administration of such a solution to a patient is merely an adjustment of particular work conditions is not correct. As a matter of fact, the efficacy of certain entarally applied compounds depends very much on gastric emptying kinetics and actual uptake from the small intestine. For this reason, it is important to administer compounds such as GTE and glutamine shortly before operation whereas other compounds such as arginine need to be given over days in order to be effective since their efficacy depends on increased blood levels.

10. I declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issuing thereon.

Signed: _____
Dr. Heinz Schneider

Date: July 21, 2007